

The following questions about Asian citrus canker and the Citrus Canker Eradication Program were provided to the Florida Department of Agriculture and Consumer Services by concerned citizens with scientific knowledge from South Florida. Scientists with the department have provided the respective answers. Additional questions or comments should be sent to Dr. Wayne N. Dixon Bureau Chief of Entomology, Nematology, and Plant Pathology, Division of Plant Industry, Gainesville, Florida. (email: dixonw@doacs.state.fl.us; fax: 352/334-0737; or mail: PO Box 147100, Gainesville, FL 32614)

Question 1a. What is the scientific evidence that *Xac* (the pathogen of citrus canker Asian strain) is deadly or otherwise devastating to citrus trees in Florida?

The adjective ‘deadly’ is not totally inaccurate, because the disease has been reported to kill mature Key lime trees in the Maldives and kill young citrus in Japan. On the other hand, ‘deadly’ does not reflect the usual outcome of Asian citrus canker on most citrus. The adjective ‘devastating’ certainly applies both in the biological and economic sense, as it reflects the manner in which the disease can lay waste to the crop and ruin its potential value.

There has never been a completely uninhibited occurrence of Asian citrus canker in Florida to establish its full potential for damage under our environmental conditions. Since the diseased trees are removed as soon as possible, no yield reduction studies have been done under such conditions. However, when Asian citrus canker has been present in an area of Florida even for a relatively short period of time before being detected and dealt with by the regulatory agencies, the escalating damage to the more susceptible cultivars of citrus is very obvious and severe. This level of damage is consistent with damage evaluations from areas with climates similar to Florida. Damage does occur even though disease control strategies are employed for other endemic disease problems.

Assessments of the economic impact of Asian citrus canker in Florida have been extrapolated from field research in Argentina (Muraro 1986, 2000). These studies enumerate several additional production expenses (copper sprays - \$56 per acre per application, 1 to several applications may be required; windbreak installation and maintenance - \$45 to \$65 per acre per year; sanitation - \$40 to \$45 per acre per year; grove and packinghouse inspection - \$30 and \$31.50 per acre per year respectively). Even with these measure in place, fresh fruit crop losses were estimated at \$80 to \$160 per acre per year for early oranges, \$31 to \$79 per acre per year for mid-season oranges, and \$69 to \$137 per acre per year for grapefruit. These figures did not include the cost of equipment to apply the pesticides in a timely manner, nor did it include the lost revenues because of quarantines on exported fruit.

Another testament to the harmful nature of Asian citrus canker can be obtained by reading a chronological sequence of basic plant pathology textbooks that cover the disease. Copies of pertinent passages from textbooks published from 1923 to 1997 are attached. A copy of the USDA’s entry on Asian citrus canker in the ‘Pests Not Known to Occur in the US’ series from 1982 is also included.

A comprehensive database of journal publications on citrus canker is available in a digital format that is searchable by key words using MS Word or MS WordPad. Some of the listings are accompanied by an abstract.

Question 1b. What is the scientific basis for treating citrus canker Asian strain differently than other cosmetic and blemishing citrus diseases?

Asian citrus canker is an exotic and damaging disease of very limited distribution in Florida. The option to eradicate still exists. This option does not exist for diseases caused by such pathogens as *Alternaria citri*, *Diaporthe citri* and *Mycosphaerella citri* because they are already well established in Florida, the US, and around the world. Furthermore, Asian citrus canker disease initiates immediate Federal quarantine measures that prohibit fresh fruit from areas where the disease is endemic being shipped to other citrus-producing areas. See the response to question 4 for further discussion of this issue.

Question 2a. What scientific methods are required for accurate detection and diagnosis of citrus canker Asian strain? (Discuss advantages and disadvantages of various methods, providing estimate of accuracy for each)?

In general, scientific methods required to identify any previously unknown plant diseases should follow Koch's rules (George N. Agrios. 1997. Plant Pathology, 4th Edition, Academic Press, NY. 635 p.) that include:

1. The pathogen must be found associated with the disease in all the diseased plants examined.
2. The pathogen must be isolated and grown in pure culture on nutrient media, and its characteristics described (nonobligate parasites), or on a susceptible host plant (obligate parasites), and its appearance and effects recorded.
3. The pathogen from pure culture must be inoculated on healthy plants of the same species or variety on which the disease appears, and it must produce the same disease on the inoculated plants.
4. The pathogen must be isolated in pure culture again, and its characteristics must be exactly like those observed in step 2.

Generally, the diagnosis of a disease may be considered complete by examining the symptoms caused by the disease (the pathogen is known to cause a particular disease syndrome) and the diagnostician is confident that no other causal agents are involved.

Asian citrus canker produces a syndrome that can be routinely detected by a citrus canker surveyor and confirmed by a well-trained plant pathologist or citrus canker diagnostician in the field. Although its appearance may vary on different varieties and under different weather conditions, Asian citrus canker generally produces raised, corky, ruptured, tan lesions with water-soaked margins and yellow haloes on citrus leaves. The symptoms of citrus canker are uniquely distinguishable from the symptoms caused by other citrus leaf pathogens, such as scab (*Elsinoe fawcettii* Bitancourt & Henkins), melanose (*Diaporthe citri* F.A. Wolf), and citrus bacterial spot (*Xanthomonas axonopodis* Starr & Garces emend. Vauterin *et al.* pv. *citrumelo* Gabriel *et al.*). Asian citrus canker can be accurately diagnosed visually by a trained field plant pathologist/diagnostician.

To avoid any possible misdiagnosis of Asian citrus canker, the Florida Department of Agriculture and Consumer Services has established a diagnostic protocol for Asian citrus canker (Table 1). Each test should render an accurate diagnosis of Asian citrus canker if carefully performed by an experienced plant pathologist or canker diagnostician. It is the protocol itself that provides an accurate diagnostic system that allows no misidentifications. Any citrus canker sample in question is sent to the Plant Disease Quarantine Facilities in Gainesville or Beltsville for a laboratory determination.

Table 1. Fourteen diagnostic protocols for Asian citrus canker employed by the Citrus Canker Eradication Program

Diagnostic Protocol	Positive Diagnosis
1) Field Examination by Survey Inspector Surveyors examine all citrus plants for lesions suggestive of Asian citrus canker, <i>i.e.</i> , raised, tiny blister-like young lesions or tan to brown old lesions with raised corky tissue in the center surrounded by water-soaked margin and a chlorotic halo. If such lesions are found on leaves, stems, or fruit, the tree is marked with white paint and placed on the list for a diagnosis by Citrus Canker Eradication Program (CCEP) Field Plant Pathologist	Suspect trees will be reported to the pathology section with CCEP
2) Field Examination by Field Plant Pathologist Field Plant Pathologist looks for typical Asian strain citrus canker syndrome on suspect trees, <i>i.e.</i> , raised, tiny blister-like young lesions or tan to brown old lesions with raised corky tissue in the center surrounded by water-soaked margin and a chlorotic halo. Diagnosis = Asian citrus canker if syndrome is present; “negative” for citrus canker if syndrome is clearly absent; or “suspect” Asian citrus canker for further lab diagnosis if syndrome is still questionable. Sample may be taken to local CCEP lab for microscopic exam (diagnostic protocol 3 and 4), or sent to the FDACS-DPI Plant Disease Quarantine Facilities in Gainesville for full diagnostic protocol (steps 3-9, steps 10-13 optional). The standard diagnostic practice calls for a full laboratory diagnosis in Gainesville on the first sample from a new section (1 sq mi area). Subsequent diagnoses for that section are routinely field diagnosed as in the first two protocols.	1st positive identification: Field diagnostician make confirmation based on the unique Asiatic citrus canker syndrome.
3) Binocular Microscope Examination Sample leaves are examined under the dissecting microscope for slightly raised, tiny blister-like young lesions or tan to brown old lesions with raised corky tissue in the center surrounded by water-soaked margin and a chlorotic halo, the symptoms of typical Asian citrus canker. If symptoms are present, diagnosis = Asian citrus canker. Samples with symptoms other than citrus canker are diagnosed using routine disease diagnosis protocol.	2nd positive identification: Asian citrus canker symptoms will show more details.
4) Compound Microscope Examination Freehand microtome cross-sections of a typical Asian canker lesion edge cut by hand with a new razor blade or scalpel blade are placed in a drop of water on a slide and covered with a cover glass. The slide is examined under the compound microscope for bacterial streaming. If bacterial streaming accompanies typical syndrome, diagnosis = Asian citrus canker.	3rd positive identification: Bacterial streaming is observed on the sample with citrus canker lesions.
5) Isolation Five to ten free-hand microtome sections of water-soaked tissue from the lesion margin are crushed in 5-10 ml of sterile tap water. The macerate is streaked onto nutrient agar (NA) plates. The plates are incubated at 28 °C for 72 hours. Single yellow and mucoid colonies (4-8) are transferred onto Lima Bean Agar (LBA) slants and incubated at 28 °C for 48 hours for typical growth of <i>Xanthomonas axonopodis</i> pv. <i>citri</i> . If typical <i>X. axonopodis</i> pv. <i>citri</i> colonies are recovered, diagnosis = Asian citrus canker.	4th positive identification: Colonies of <i>Xanthomonas</i> are present on nutrient medium agar plate.
6) ELISA test (optional) The macerated lesion tissues from diagnostic protocol 5 may be utilized for an ELISA test (using Agdia’s kit) with a suitable positive and negative control. If positive test obtained, diagnosis = Asian citrus canker.	5th positive identification: Yellowish color appears in the testing wells.

<p>7) Recovery of the Bacterium from Old or Otherwise Difficult Lesions To insure a successful isolation of the causal bacterium from the lesion, the macerate of the crushed lesions (obtained as in Protocol 5 above) is infiltrated into the intercellular space of Duncan grapefruit leaves. Lesions appearing 4-6 days after inoculation on those inoculated leaves are used in the process of isolation. If typical lesions appear on the inoculated plant, and typical <i>X. axonopodis</i> pv. <i>citri</i> colonies are recovered from those lesions, diagnosis = Asian citrus canker.</p>	<p>6th positive identification: Canker lesions appear 6-10 days after inoculation.</p>
<p>8) Pathogenicity Test Each culture of selected isolates of <i>Xanthomonas axonopodis</i> pv. <i>citri</i> on LBA slants is diluted to 10⁸ CFU/ml and 10³ CFU/ml. The bacterial suspensions are infiltrated using a new blunt syringe (without needle) into the leaves of Duncan grapefruit, Swingle citrumelo, 'Madame Vinous' sweet orange or Key lime for determination of their pathogenicity. The causal nature of <i>X. axonopodis</i> pv. <i>citri</i> is confirmed if the typical symptoms appear 5-10 days after infiltration. If symptoms appear, diagnosis = Asian citrus canker.</p>	<p>7th positive identification: Canker lesions appear 6-10 days after inoculation.</p>
<p>9) Hypersensitive Reaction on Non-host Plant The bacterial suspension of each isolate(at 10⁸) is infiltrated into leaves of tomato, tobacco and pepper for determination their hypersensitive reaction (HR). <i>X. axonopodis</i> pv. <i>citri</i> usually causes HR positive on tomato, and negative on tobacco and pepper. If the typical HR reactions occur, this supports a diagnosis of Asian citrus canker (the test alone is not diagnostic, it only lends support to the diagnosis).</p>	<p>8th positive identification: Brownish color appears on tomato, but not on pepper and tobacco 24-48 hours after infiltration.</p>
<p>10) PCR-based Assay The DNA of the suspect citrus canker bacterium from either fresh lesions or pure culture is extracted, amplified using a specific primer for detecting <i>X. axonopodis</i> pv. <i>citri</i>, separated in an agarose gel, and stained.</p>	<p>9th positive identification: The target band appears in the testing gel.</p>
<p>11) Fruit Lab Facility with USDA, ARS in Beltsville, MD (optional) The ARS Fruit Lab (Dr. John Hartung, Group and Project Leader) has the following capabilities: 1) isolation, purification, pathogenicity test of <i>X. axonopodis</i> pv. <i>citri</i>; 2) Enzyme-Linked Immunosorbent Assay (ELISA); 3) plasmid-based hybridization probes; and 4) PCR-based assay. All these techniques are available for the rapid, specific and sensitive detection of <i>X. axonopodis</i> pv. <i>citri</i>. Positive results with any or all of these tests, diagnosis = Asian citrus canker.</p>	<p>10th positive identification</p>
<p>12) Agdia, Inc. (optional) Agdia, Inc. testing services in Elkhart, IN has the capacity to diagnose <i>X. axonopodis</i> pv. <i>citri</i> using ELISA. This option is utilized in special cases when an independent second diagnosis is requested by the citrus owners and permitted by the CCEP and FDACS-DPI Directors. With a positive ELISA test, diagnosis = Asian citrus canker.</p>	<p>11th positive identification: Yellowish color appears in the testing wells.</p>
<p>13) Department of Plant Pathology, University of Florida - Gainesville (optional) Dr. Dean Gabriel's laboratory uses a specific DNA probe to detect <i>X. axonopodis</i> pv. <i>citri</i>. If the probe detects the specific DNA sequence unique to <i>X. axonopodis</i> pv. <i>citri</i>, diagnosis = Asian citrus canker. The protocol is used in the FDACS - Advanced Diagnostics Laboratory as well.</p>	<p>12th positive identification: Target DNA band appears in the testing gel.</p>

14) Department of Plant Pathology, University of Florida - Gainesville (optional) Dr. Jeff Jones's lab does MIDI profiling / fatty acid analysis to confirm <i>X. axonopodis</i> pv. <i>citri</i> . If the fatty acid profile matches that in the library for <i>X. axonopodis</i> pv. <i>citri</i> isolates, diagnosis = Asian citrus canker.	13th positive identification: Similarity coefficient of the isolate matches that of the A-strain citrus canker.
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Question 2b. What is the incubation period of the bacterium?

The time interval from infection of a plant to appearance of disease symptoms on the plant is called the incubation period. In natural infections by bacterial plant pathogens, incubation periods are generally 5-7 days with a few exceptions. The length of the incubation period is governed by various factors of the pathogenic bacteria (species, mode of entry, inoculum density, and type of disease), host plant (species, age, and organ), and environment (humidity and temperature).

Under optimum weather conditions and with 10^8 cells for an artificial inoculation site, it takes about 5-7 days for Asian citrus canker bacterium to produce the symptom on the succulent young leaves of a susceptible citrus variety. When infection occurs late in fall, the bacteria as well as host tissue quickly become dormant because of the low temperatures. Consequently, the incubation period is extended to the following spring, and therefore resulting in a significant latent infection.

Question 2c. Which plants serve as hosts?

In general, all species in the genus *Citrus* and *Poncirus* serve as hosts of Asian citrus canker. This is particularly true in Florida with the evidence in three eradication programs. A number of plants in the *Rutaceae* other than *Citrus* and *Poncirus* can serve as hosts of *X. axonopodis* pv. *citri* under experimental conditions or heavy disease pressure in nature.

The *Citrus spp.* and the following hybrids listed below are natural hosts of *X. axonopodis* pv. *citri*, with varying degrees of susceptibility to the pathogen. In addition to the species of host plant, susceptibility is also affected by the plant part affected, whether leaves, fruits or twigs. It is important to recognize that other than *Citrus spp.* and their hybrids, most plants, except *P. trifoliata*, are not sufficiently susceptible to *X. axonopodis* pv. *citri* under natural conditions to warrant attention as hosts of the bacterium. It is likely that the less than common hosts of Asian citrus canker are less frequently found in the residential landscape. The CCEP will review data that may suggest a change in risk assignment of a potential host and adjust regulatory strategies as warranted. The recognition of plants that may serve as hosts of Asian citrus canker is an important aspect of the training received by CCEP surveyors and plant pathologists.

Common hosts:

Citrus spp.
Citrus aurantiifolia (lime)
Citrus aurantium (sour orange)
C. deliciosa x *C. maxima* (Tangelo)
Citrus grandis (pummelo)
Citrus hystrix
Citrus junos (yuzu)
Citrus limetta (sweet lemon tree)
Citrus limon (lemon)

Citrus medica (citron)
Citrus madurensis (calamondin)
Citrus natsudaiddai (natsudaiddai)
Citrus x paradisi (grapefruit)
Citrus reshni (cleopatra mandarin)
Citrus reticulata (mandarin)
Citrus x Poncirus trifoliata (citrumelo)
Citrus sinensis (navel orange)
Citrus sunki (sour mandarin)
Citrus tankan
Citrus unshiu (satsuma)
Eremocitrus glauca (Australian desert lime)
Limonia acidissima (elephant apple)
Poncirus trifoliata (Trifoliate orange)
Fortunella japonica (round kumquat)
Fortunella margarita (oval kumquat).

Less common hosts:

C. aurantiifolia x *Microcitrus australasica* (Faustrime)
C. limon x *M. australasica* (Faustrimon)
C. madurensis x *M. australasica* (Faustrimedin)
C. sinensis x *Poncirus trifoliata* (Citrangle)
C. aurantiifolium x *P. trifoliata* (Citradia)
C. nobilis x *P. trifoliata* (Citrandin)
C. unshiu x *P. trifoliata* (Citrushu)
Citrangle x *P. trifoliata* (Cicitrangle)
C. adurensis x *Citrangle* (Citrangedin)
C. deliciosa x *Citrangle* (Citrangarin)
C. unshiu x *Citrangle* (Citranguma)
Fortunella margarita x *Citrangle* (Citranglequat)
F. japonica x *C. aurantiifolia* (Limequat)
C. maxima x *C. aurantiifolia* (Limelo)
C. madurensis x *C. aurantiifolia* (Bigaraldin)
C. maxima x *C. sinensis* (Orangelo)
F. margarita x *C. sinensis* (Orangequat)
C. nobilis (Clementine) x *C. maxima* (Clemelo)
C. nobilis (King of Siam) x *C. maxima* (Siamelo)
C. unshiu x *C. maxima* (Satsumelo)
C. nobilis (King of Siam) x *C. sinensis* (Siamor)
C. deliciosa x *C. madurensis* (Calarin)
C. unshiu x *C. madurensis* (Calashu)
Aegle marmelos (golden apple)
Casimiroa edulis (white sapote)

Plants other than *Citrus* spp.:

The following plants have also been reported as susceptible to *X. axonopodis* pv. *citri*, however, the original descriptions were either unconfirmed (U) or contradict those of other authors (C):

Aegle marmelos (C)
Balsamocitrus paniculata (U)
Feroniella obligata (U)
Matthiola incana var. *annua* (U)
Toddalia asiatica (C)
Ageratum conyzoides (U)

Finally, Peltier and Frederick (1920, 1924) (see below), who defined susceptibility on the basis of artificial inoculation in the greenhouse or field, listed additional potential hosts. These two articles are being reviewed to better understand the nature of the experimental design. Once completed, the results of the review will be posted to this web site.

Peltier, G.L. 1918. Journal of Agricultural Research 14: 337 – 357.

Pelter, G.L., and W.J. Frederick. 1920. Journal of Agricultural Research 19: 339 – 362.

Pelter, G.L., and W.J. Frederick. 1924. Journal of Agricultural Research 28: 227 – 239.

Question 2d. What is the accepted methodology for establishing age of lesions?

Age of citrus canker lesions on a citrus tree can be estimated based on their appearance on leaves, fruit and stems. To determine the oldest lesion age, the plant pathologist/diagnostician must have knowledge of symptom development of Asian citrus canker on various parts of citrus and experience in differentiating lesion appearances at the different stages on different varieties. Lesion size, color and extent of necrotic tissue, presence of halo around the lesion, secondary fungal infection on lesions, fruit lesion (time of lesion appearance and period of fruit susceptibility), and stem lesion appearance (on which growth flush and the number of flushes back, etc.), are some factors typically considered when the lesion age is estimated.

Examples of different Asiatic Citrus Canker Lesion Ages



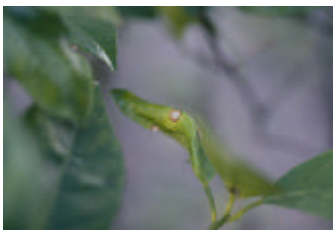
Two weeks old lesions



One month old lesions



Three months old lesions



4-5 months old lesions



6-8 months old lesions

Question 3a. What inspection programs are in effect in Indian River counties and the Ridge? (Provide details of frequency, methods of inspection, personnel involved and their scientific credentials etc.)?

Statewide canker survey of citrus groves is in progress. Approximately 830,000 acres of citrus groves will be surveyed once a year in Florida by 275-300 well-trained canker inspectors along east, west coastal and central Ridge citrus-producing areas. Survey priority is given to the groves where grapefruit and some susceptible sweet oranges are the major cultivars.

Citrus canker grove inspectors visually examine each citrus tree bed by bed either by foot (in a small grove) or on an All Terrain Vehicle (ATV) (in a large grove) looking for suspect symptoms of citrus canker. Any canker suspect sample is submitted to the Plant Disease Quarantine Facilities in Gainesville for further confirmation. A positive laboratory confirmation of Asian citrus canker will result in an immediate action to remove diseased trees and the adjacent or exposed citrus trees within 1900 feet.

All citrus grove inspectors, like citrus residential inspectors, are recruited with a minimum requirement of a high school diploma. They attend special training before being dispatched to the field. The training includes in-class and in-field recognition of citrus canker and other citrus diseases, determination of citrus varieties and sample collecting procedures. They are required to submit any suspicious samples to the Plant Disease Quarantine Facilities in Gainesville, which is led by Drs. Xiaolan Sun and Tim Schubert for an accurate citrus canker diagnosis.

Question 3b. How do these inspection programs compare with those in Dade and Broward counties?

Dade and Broward counties are currently under citrus canker quarantine. A more frequent survey schedule is in effect in the areas where Asian citrus canker has been found and confirmed (Dade, Broward, Palm Beach, Collier, Hendry, Manatee, Hillsborough counties). A 30-day, 60-day, 90-day, 6-month, or one-year schedule is established depending on disease conditions, resources, and other factors. The canker suspect trees will be confirmed visually in the field plant pathologist/diagnostician. A sample from the first diseased trees found in an area of one square mile is submitted to the Plant Disease Quarantine Facilities in Gainesville for a pathogenicity test.

Question 3c. How many groves are thoroughly inspected on a regular basis?

About 700,000 of 832,250 acres of commercial citrus groves have been thoroughly inspected for citrus canker. The goal of the CCEP is to inspect annually all commercial citrus acreage and with greater frequency any commercial citrus groves within a quarantined area. Prior to the CCEP, survey objectives were for just 25% of all groves inspected annually so that 100% of the groves would be inspected every four years.

Question 4. Why is it necessary to eradicate citrus canker if the main reason for the program is so that fruit can be exported? The Department of Citrus ran tests at the request of the citrus industry in 1985 or 1986 showing that there were several chemicals that could completely disinfect the fruit

(quaternary ammonium compounds were one group). Fruit treated with these chemicals was exported to countries maintaining quarantines on citrus canker and the fruit was acceptable. The quaternary ammonium compounds are commonly used for disinfection of equipment and vehicles in groves. Please comment.

Asian citrus canker causes direct damage to citrus plants and fruit, whether that product is destined for export or not. Asian citrus canker damages citrus both in the commercial and residential setting. Eradication is deemed a worthwhile goal because the overall costs of trying to manage the disease year after year are estimated to be much higher than the cost of eradication. The eradication of Asian citrus canker will benefit all citrus growers, whether commercial or private.

It is true that one of the main impacts of Asian citrus canker could be the loss of export markets for fresh fruit. The Florida Department of Agriculture & Consumer Services – Division of Plant Industry, the Florida Department of Citrus, and the USDA-APHIS-PPQ have all been instrumental in addressing the issue of safe export of fresh fruit from Asian citrus canker quarantine areas. Since there is some legitimacy to the quarantines established by citrus-producing areas that do not have Asian citrus canker, this issue will likely take time to resolve to the satisfaction of all stakeholders.

The studies referenced in the question above were done in 1986 and resulted in the publication of a journal article (Brown and Schubert, Plant Disease 71: 319-323, 1987). The test organism in those studies was actually the tomato bacterial leaf spot pathogen (*Xanthomonas axonopodis* pv. *vesicatoria*), which was used as a surrogate for the citrus bacterial spot (CBS) organism (*Xanthomonas axonopodis* pv. *citrumelo*). The surrogate was used to avoid the constraints of conducting the experiments under BL-2 quarantine containment conditions, which was a federal and state requirement for working with the CBS bacterial pathogen at that time. No *X. axonopodis* pv. *citri* was used. SOPP (sodium orthophenyl phenate) and chlorine were the main products investigated as fruit surface disinfectants. Other disinfectants investigated were dual chain quaternary ammonium disinfectants, chlorine dioxide, and peracetic acid. At the time of the study, quaternary ammonium products were not approved for use directly on food products, and that remains the case today. All others presently have government approval for sanitizing food surfaces. Even though the experiments did not actually test the efficacy of surface disinfectants against *X. axonopodis* pv. *citri*, there are sound scientific reasons to think that the results derived should be essentially identical. None of the products was able to completely disinfect (in the strict sense of the word) the artificially contaminated fruit surface every time, but the inoculum reduction was deemed sufficient to eliminate the risk of transmitting inoculum to a new area at a level high enough to permit establishment.

More recently, the Citrus Canker Risk Assessment Group has prepared a report entitled “Bacterial Citrus Canker and the Commercial Movement of Fresh Citrus Fruit.” The report presents a risk analysis assessing the likelihood of *X. axonopodis* pv. *citri* inoculum moving to and establishing in another citrus-producing area on the surface of symptomless citrus fruit. The step by step review of the process from grove to final destination makes a strong case for using grove and packinghouse inspection coupled with various treatments on the packinghouse line to effectively eliminate the risk of transmitting *X. axonopodis* pv. *citri* inoculum via fresh fruit. The proposal is slated for publication in the Federal Register to allow comments from stakeholders in the citrus fresh fruit industry. However, this would only be acceptable to our export market provided Florida continued in an active eradication program. If the disease becomes established it’s highly unlikely that shipment of symptomless fresh fruit from a quarantine area would ever be permitted.

Question 5a. What is the scientific evidence that justifies the cutting of healthy citrus trees and, in particular, all citrus trees within a 1900-foot radius of an infected tree?

A Global Positioning Satellite-based census study monitored over 19,000 healthy and diseased dooryard citrus trees in four study areas established in Miami-Dade and Broward cos. This 18-month epidemiological study was instrumental for establishing that disease gradients resulting from bacterial dispersal occurred over distances in excess of 125 feet. The study was designed and implemented by plant pathologists / epidemiologists associated with the USDA, Agricultural Research Service; University of Florida, Citrus Research and Education Center; University of Edinburgh, Institute of Ecology and Resource Management, Scotland; and Florida Department of Agriculture and Consumer Services, Division of Plant Industry.

Trees in this study were assayed for disease severity, age of the oldest infection, direction and location of infection, citrus cultivar, tree age, and canopy size. The physical location of each tree was determined by differential GPS and its coordinates were recorded. All trees in each study area were re-surveyed approximately every two months. For each study site, distance measurements between focal trees and newly infected trees were calculated for various temporal windows of 30, 60, 90, and 120-days in duration, corresponding to intervals of inspector survey. A Visual Basic routine was used to calculate the distances between each newly diseased tree and all prior focal trees. The nearest distance was used because it was considered to be the most conservative estimate possible. It is therefore likely to be an underestimate of spread, but is a good estimate of the minimum possible distances of spread. Distances of spread were parsed into consecutive 15-meter (50-ft) distance categories and plotted as frequency distributions via a second Visual Basic routine.

In December 1998, results of the epidemiological study were reviewed by a group of scientists and regulatory officials. The press was also invited and a reporter for a major Central Florida newspaper was in attendance for a portion of the meeting. The meeting was not secret. The consensus was: 1) the 125 ft (38 m) radius used to define exposure was inadequate to suppress the spread of canker and 2) although disease spread was detected up to 58,850 ft (17942 m), the majority of new canker infections occurred within about 1900 ft (579 m) of known source trees. The spread from rainstorm and other events was measured up to 1200 feet (90%), 1900 (95%), and 2700 feet (99%) from known infected disease source trees. The percentages represent the proportion of infected trees located within the indicated radius. As a result, a new regulation, the '1900 ft rule', was put into practice in January 2000, requiring the removal and destruction of diseased citrus trees and of all citrus trees within a 1900-ft radius of a diseased tree.

This work has been specifically reviewed by a world-recognized plant pathologist/epidemiologist at Ohio State University who concurred with the findings of the study. It was also reviewed anonymously by three other plant pathologists with epidemiological knowledge and by the senior editor of *Phytopathology* prior to the first publication.

In addition, field data and observations have been collected from more than 25 commercial citrus groves during the current citrus canker infestation in Florida. The spread of disease in these groves, in terms of numbers of infected trees and distances between trees, further substantiates the long distance dispersal of the bacteria.

T. Gottwald, X. Sun, T. Riley, and J. Graham. 1999. Estimating spread of citrus canker in urban Miami via GPS. (Abstr.) *Phytopathology* 89 (suppl.): S29. (Annual Meeting of the Association of Phytopathological Society, Montreal Canada).

T. Gottwald, X. Sun, T. Riley, J. Graham and G. Hughes. 2000. Estimating Spread Of Citrus Canker In Urban Miami Via Differential GPS. International Citrus Canker Research Workshop June 20-22, 2000, Ft. Pierce, Florida. URL: <http://doacs.state.fl.us/~pi/workshop.html>.

T. Gottwald, J. Graham, D. Egel. 1992. Analysis of foci of Asiatic citrus canker in a Florida citrus orchard. *Plant Disease* 76: 389-396.

Question 5b. What methods were used to verify the source of bacterial infection in the 1900 ft study?

The GPS-based census study of citrus trees was focused on determining the shortest distance from a focal positive (citrus canker-infected tree with oldest infestation and highest disease severity) tree to the newly detected secondarily infected tree. These calculated distances would result in a conservative estimate of bacteria dispersal distances. From the outset of the study, the investigators recognized that citrus canker-infected trees from further away and in any direction may contribute to new infections in any of the study sites, but the inoculum from those trees would render a longer distance calculation than that of the focal trees. Survey by the Citrus Canker Eradication Program was conducted on an operational basis to detect any citrus canker-infected trees outside each study site.

Current analyses are investigating the influence of wind direction and rainfall on the observed disease spread in each of the four study sites.

Question 5c. Why will the citrus canker eradication program not consider disinfection methods that are likely to yield the same probability for infection as the 1900-ft. rule (i.e. 95% effective)?

More accurate terms than disinfection would be sanitation or decontamination as it occurs in the Citrus Canker Eradication Program. The lack of a well-controlled environment, i.e., out in the field, suggests that “sanitation” more accurately describes the intended elimination of bacterial cells from topical surfaces (not diseased plant tissues). Chemicals used for sanitation are not designed for use on plant tissues and have been proved ineffective for such purposes. Also, it is not accurate to assume that sanitation methods are equivalent to the 1900-ft rule. The 1900-ft only describes the majority of new canker infections that occurred within about 1900 ft (579 m) of known source trees. The spread from all dispersal methods was measured up to 1900 (95%), and 2700 feet (99%) from known infected disease source trees. The percentages represent the proportion of infected trees located within the indicated radius.

Question 5d. A discussion of the eradication program by Gottwald *et al.* was recently published as a Letter to the Editor in *Phytopathology*. This letter refers to results of the 1900 ft study, but does not provide details of experimental design, methodology, etc. Where and when will the details of this study be published and when will the original data be available to the public?

It is the intention of the GPS-based census study scientists to submit a manuscript to the refereed journal *Phytopathology* later this year. The manuscript may be published late this year or early 2002 depending on the manuscript's submission date and then successful acceptance by the review panel and editors of this science journal. This manuscript will describe, as required by the journal, the materials and methods and contain a results section and listing of literature cited. The data that will form the basis of this manuscript are in the final stages of in-depth analysis by the study's investigators. To date, these analyses, as they have been completed, are evidencing no changes to previously presented or discussed findings.

Additional manuscripts are anticipated, perhaps two or three more on other epidemiological aspects of citrus canker in an urban environment. Once these manuscripts are similarly submitted to an appropriate refereed journal and accepted, then the foundation data set may be made available to the public.

Scanned images of several documents have placed on our web site to provide additional background information. (You will need to go the web site as listed on our web page to get the free plug-in for Netscape Navigator and Internet Explorer. In the case of the eight files listed below, we have used the file compression protocol to reduce the overall file size from 8 Mb to that of 1 Mb.)

These documents are:

Agrios, G.N. 1977. Plant pathology. 4th Ed. Academic Press, San Diego. pp. 445 - 449.

Anonymous (USDA). 1982. Pests not known to occur in the United States or of limited distribution, No. 27: Citrus canker. USDA, APHIS, PPQ. Hyattsville, MD. 12 p.

Batchelor, L.D., and H.J. Webber. 1948. The citrus industry: Production of the crop. University of California Press, Berkeley. Volume 11: 526 - 529.

Brown, G.E., and T.S. Schubert. 1987. Use of *Xanthomonas campestris* pv. *vesicatoria* to evaluate surface disinfectants for canker quarantine treatment of citrus fruit. Plant Disease 71: 319 – 323.

Fawcett, H.S. 1936. Citrus diseases and their control. McGraw-Hill Book Co., Inc. pp. 237 - 249.

Goto, M. 1992. Fundamentals of bacterial plant pathology. Academic Press, Inc., San Diego. pp. 315 - 319.

Hume, H.H. 1957. Citrus fruits. The Macmillan Co. New York. pp. 390 - 395.

Wheeler, H.J. 1923. Citrus culture in Florida. The American Agricultural Chemical Co. pp. 78 - 79.